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A set of primers for plastid indels and nuclear microsatellites in the invasive plant *Heracleum mantegazzianum* (Apiaceae) and their transferability to *Heracleum sphondylium*

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Abstract

This study reports the isolation and polymorphism characterization of four plastid indels and six nuclear microsatellite loci in the invasive plant *Heracleum mantegazzianum*. These markers were tested in 27 individuals from two distant *H. mantegazzianum* populations. Plastid indels revealed the presence of five chlorotypes while five nuclear microsatellite loci rendered polymorphism. Applications of these markers include population genetics and phylogeography of *H. mantegazzianum*. A very good transferability of markers to *Heracleum sphondylium* was demonstrated.

Keywords: biological invasions, indels, microsatellites, plastid DNA

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The giant hogweed (*Heracleum mantegazzianum* Sommier & Levier; Apiaceae) is a perennial monocarpic diploid weed that successfully invaded over 20 European countries (Tiley *et al*. 1996). It reproduces exclusively sexually and a single plant can produce up to 50 000 seeds (Pyšek *et al*. 2007). The ecology of giant hogweed has been thoroughly studied, yet its population genetics has lagged behind with only two studies up to date (Walker *et al*. 2003; Jahodová *et al*. 2007). Here, we describe the isolation and polymorphism characterization of four plastid indels and six nuclear microsatellite loci which may be useful for population genetic and phylogeographical studies of the species. We also tested the transferability of loci to a common close relative (*Heracleum sphondylium* L.) which is naturally present in the invaded range.

Hydrochory is believed to represent an important mechanism of the spread of *H. mantegazzianum*. Consequently, markers specific to the chloroplast genome, which are generally transmitted maternally in angiosperms, represent potentially useful tools to study seed-mediated gene flow. Plastid indels were identified by sequencing the *trn*T-*trn*L intergenic spacer (following Taberlet *et al*. 1991) on 12 *H. mantegazzianum* individuals from different locations. Sequencing was performed using Big Dye 3.1 Terminator cycle sequencing kit (Applied Biosystems) according to manufacturer's instructions and an ABI PRISM 3100 genetic analyser (Applied Biosystems). Primer sequences were then defined in regions flanking the four identified indels (Table 1).

Microsatellite sequences were isolated from polymerase chain reaction (PCR) products of intersimple sequence repeat (ISSR) fragments. Individual ISSR-PCR was carried out using six 5′-anchored microsatellite primers that gave clear multiband products in an initial screen in *H. mantegazzianum*: $GGCC(AG)_8$, $GGCC(AC)_8$, $CCGG(AG)_8$, $CCGG(AC)_8$, $GCGC(AG)_8$ and $GCGC(AC)_8$. Reactions were carried out in a total volume of 20 µL containing 100 ng genomic DNA, 20 pmol primer, 1× PCR buffer, 200 µM each dNTP, 2.5 mM $MgCl_2$ and 1 U *Taq* polymerase (Genetix) using the following parameters: initial denaturation at 94 °C for 3 min followed by 35 cycles of denaturation at 94 °C, annealing at 60 °C for 1 min, extension at 72 °C for 1 min and a final extension at 72 °C for 5 min. Ten microlitres of PCR product was resolved on a 2% agarose gel and visualized by ethidium bromide staining to ensure clarity

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Table 1 Characteristics of *trn*T-*trn*L indels (plastid DNA) and nuclear microsatellites for two populations of *Heracleum mantegazzianum* and *Heracleum sphondylium* including fluorochrome used, GenBank Accession nos, primer sequences, microsatellite motif (repeat type), annealing temperature (T_a; in °C) and allele size range (in bp)

Locus	Fluorochrome	GenBank no.	Primers	Repeat type	T_a	Allele sizes	
						H. mantegazzianum	*H. sphondylium*
Plastid DNA							
*trn*T-L indel1	HEX (green)	AM493757-AM493768	F. *AGATAAATTCTACCTGCAAGG R. TGACTAGCTAATAGTAATCGC	—	56	84, 90	84, 90
*trn*T-L indel2	HEX (green)	AM493757-AM493768	F. *TTAGTTTTTTTCTCACATCAC R. GATTTAATCTAAAAATAGAAC	—	53	98, 107	98, 107
*trn*T-L indel3	NED (yellow)	AM493757-AM493768	F. *TCCATCTTTACGAATCAAAG R. ATTACACTTCTATATTTTATTGC	$(TA)_7(T)_7$	47	110, 112, 117	110, 112
*trn*T-L indel4	HEX (green)	AM493757-AM493768	F. *TTCTGATTGGACCAAATGCG R. TCTACCGATTTCGCCATATC	—	53	214	147, 214
Nuclear DNA							
HMNSSR131	NED (yellow)	EF654668	F. *GCGATTCTCGATCTGTAAGCTT R. TACTATAATTCTGAACCCTAGTT	$(TA)_8$	57	124–134	124
HMNSSR132A	FAM (blue)	EF654669	F. *CGATTGCTCTTCTTTTGAGCAT R. AGGGTTTTGATAAAGTTAGGAAT	$(CT)_7$	58	113–115	107–113
HMNSSR132B	NED (yellow)	EF654669	F. *ATTCCTAACTTTATCAAAACCCT R. AGAGAGCCAGGTTTTGTATAAC	$(CT)_7$	58	93–103	88–95
HMNSSR140	NED (yellow)	EF654670	F. *GTATCCGGATCTGTACCTGTA R. GCCTACAAAATCAAACAACTGA	$(TC)_8$	58	122–146	120–152
HMNSSR206	FAM (blue)	EF654671	F. *GCGATTGCTCTTCTTTTGAGCA R. TTGGGGTTTTGATAAAGTTAGGAA	$(TC)_{10}$	58	116–118	110–116
HMNSSR211	HEX (green)	EF654672	F. *CAGCCTTCTTGTTGTTATCACCA R. TGGGTGTTAGAGTTTTGAAAAAGA	$(CT)_8$	58	115	117–121

*Primer labelled with a fluorochrome.

of fragments. The remaining 10 μL from each reaction was purified, pooled and ligated into pGEM-T (Promega) according to manufacturer's instructions. Following transformation into *Escherichia coli* JM109, 48 positive (white) clones were sequenced. All clones had inserts containing flanking microsatellite motifs corresponding to the anchored primer used to generate the ISSR fragment. Nine unique clones contained one internal microsatellite motif. For these, pairs of flanking primers were designed to amplify the repeated regions.

The developed markers were tested on 27 *H. mantegazzianum* individuals sampled in two geographically distinct locations: the western Swiss Alps (46°35′17″N, 7°03′68″E ; invaded range) and the Caucasus Mountains of southwest Russia (44°17′15″N, 38°54′04″E; native range). In addition, 12 *H. sphondylium* individuals from two Swiss populations [(i) 46°44′70″N, 7°94′86″E; (ii) 46°33′94″N, 7°92′10″E] were also analysed. PCR was performed using a Biometra T3 Thermocycler in a total volume of 25 μL containing 1× reaction buffer (Go*Taq* reaction buffer; 1.5 mM $MgCl_2$, pH 8.5; Promega), 200 μM of each dNTP, 0.2 μM of each primer, template DNA (100 ng) and 1 U Go*Taq* DNA polymerase (Promega). Each forward primer was labelled with one fluorescent dye (Table 1). A ratio of half-labelled to half-unlabelled forward primer was used in order to attain optimal amplifications. Cycling parameters were as follows: one cycle at 94 °C followed by 35 cycles of 45 s at 94 °C, 1 min at annealing temperature (Tables 1 and 2), 2 min at 72 °C, and a final cycle at 72 °C for 10 min. PCR products were multiplexed on an ABI PRISM 3100 with

Table 2 Number of alleles (N_a), average observed (H_O) and expected (H_E) heterozygosities at the six nuclear microsatellites for two populations of *Hercaleum mantegazzianum* and *Hercaleum sphondylium*

Locus	*H. mantegazzianum*			*H. sphondylium*		
	N_a	H_O	H_E	N_a	H_O	H_E
HMNSSR131	5	0.593	0.620	1	—	—
HMNSSR132A	3	0.184*	0.354	2	0.667	0.400
HMNSSR132B	6	0.431	0.447	3	0.500	0.333
HMNSSR140	2	0.107	0.099	8	0.667	0.592
HMNSSR206	2	0.148*	0.321	2	0.583	0.333
HMNSSR211	1	—	—	3	0.500	0.408
Total		0.293	0.368		0.583	0.413

*Significant excess of homozygozity ($P < 0.001$).

GeneScan-350 size standard, and alleles were identified using GENEMAPPER version 3.7 (Applied Biosystems).

For microsatellites, the software FSTAT 2.9.4. (Goudet 2005) was used to test for linkage disequilibrium, deviation from Hardy–Weinberg equilibrium, as well as observed and expected heterozygosities, number of alleles and allele size range. The software MICRO-CHECKER 2.2.3. (van Oosterhout *et al*. 2004) was used to check for the presence of null alleles.

The combined use of the four plastid loci developed here (Table 1) enabled the identification of five chlorotypes in *H. mantegazzianum* while four chlorotypes were detected in the sample of *H. sphondylium*. In *H. mantegazzianum*, four chlorotypes were found in the native population, and an additional one in the invasive population. No chlorotypes were shared between the two populations, confirming strong differentiation among the two samples. The combination of these loci may be useful to follow pattern of seed dissemination in *H. mantegazzianum*.

Of the nine primer pairs tested to amplify microsatellites, six produced reliable amplifications and five gave polymorphism in *H. mantegazzianum* (Table 2). The number of alleles ranged from two to six, and average observed and expected heterozygosities were 0.293 and 0.368, respectively. All populations sampled here did not differ significantly from Hardy–Weinberg equilibrium, yet a significant linkage disequilibrium was detected between loci HMNSSR132A and HMNSSR206, even at the 1% nominal level. The possible presence of null alleles was detected in the same two loci (HMNSSR132A and HMNSSR206; Table 2). A successful amplification of the microsatellite loci was obtained in *H. sphondylium* and five of them gave polymorphism. In contrast to *H. mantegazzianum*, locus HMNSSR131 was not polymorphic but three alleles were found at the monomorphic locus HMNSSR211. Applications of these markers include population genetics and phylogeography of *H. mantegazzianum* as well as *H. sphondylium*. Furthermore, the loci described here could be useful in detecting interspecific hybrids between the two species used here as well as with other *Heracleum* species.

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